REMARKS/ARGUMENTS

The Invention

The invention relates to polypeptides having PhzO activity which are encoded by nucleic acids of defined structure.

Status of the Claims

Claims 8 and 9 are rejected under 35 U.S.C §112, First Paragraph.

Amendments to the Claims

Claims 8 and 9 are amended to improve clarity and to expedite prosecution of the application. No new matter is added.

Support for the Claim Amendments

Support for the amendment to Claim 8(d) and Claim 9(b), wherein a polypeptide having an amino acid sequence with at least 95% sequence identity with SEQ ID NO:2 is recited, can be found *e.g.,* in paragraph 134, line 4 of the specification.

Support for the amendment to Claim 8(f) can be found in original Claim 9.

IDS

Applicants thank the Examiner for making the extra effort to obtain and review the Mavrodi et al. (AH1) reference.

Response to Rejections Under 35 U.S.C §112 First Paragraph, Enablement

Claims 8 and 9 are rejected under 35 U.S.C §112 First Paragraph for alleged failure to meet the enablement requirement. Applicants traverse the rejection.

The Examiner alleges that: "The specification does not teach a polypeptide encoded by a sequence 50% or 60% identical with SEQ ID NO:1 nor one that hybridizes under medium stringency, nor a subsequence of this nucleotide, nor a polypeptide having 60%

sequence identity to SEQ ID NO:2 nor a polypeptide encoded by a subsequence of a nucleotide of at least 100 nucleotide of the polynucleotide encoding the SEQ ID NO:2." The Examiner has indicated that he would allow claims to 95% sequence identity.

To satisfy the enablement requirement, an application must contain sufficient information regarding the subject matter of the claims so as to enable one skilled in the art to make and use the claimed invention. MPEP 2164.01. The test for enablement is set forth in *In re Wands*, 858 F.2d 731, 8 USPQ 2d 1400 (Fed. Cir. 1988), and requires consideration of multiple factors including: the breadth of the claims; the nature of the invention; the state of the prior art; the level of predictability in the art; the amount of direction provided by the inventor; the existence of working examples; and the quantity of experimentation needed to make or use the invention based on the content of the disclosure.

In the present case, the claims are directed to isolated polypeptides having readily testable activity which are encoded by nucleic acids with a defined structure. Working examples are provided. The specification contains ample directions to practice the invention, teaching for example, methods of cloning nucleic acid sequences (*see e.g.*, paragraph 69), sequencing (*see e.g.*, paragraph 223), determining percent sequence identity (*see e.g.*, paragraphs 71 and 72) transformation and selection (*see e.g.*, paragraphs 197-206 and 189-195), assays for metabolite identity (*see e.g.*, paragraphs 229 and 230) and assays for activity (*see e.g.*, paragraphs 69 and 231).

The level of technical sophistication is high in the art. Although some experimentation may be necessary to distinguish nucleic acids having with 50% sequence identity to SEQ ID NO:1 which encode polypeptides having PhzO activity, from all proteins that could, in principle, be encoded by nucleic acids having 50% sequence identity to SEQ ID NO:1, such experimentation utilizes well-established techniques and is routinely conducted in the art. Thus, such experimentation does not constitute undue experimentation. MPEP §2164.01.

The specification teaches nucleotide sequences that hybridize under medium stringency to SEQ ID NO:1

The Examiner alleges that the specification does not teach a polypeptide encoded by a nucleic acid sequence that hybridizes under medium stringency conditions. Applicants respectfully disagree with this allegation.

A patent need not teach, and preferably omits that which is well known in the art MPEP 2164.01. In paragraph 75 of the specification, Applicants teach that nucleic acid probes to identify and clone DNA that encodes polypeptides having the desired enzyme activity can be prepared according to methods well known in the art. These probes can then be used for hybridization with the genomic or cDNA of the genus or species of interest following standard Southern blotting procedures.

In paragraphs 76 through 79 Applicants teach specific hybridization conditions, and refer to the standard laboratory manual, Sambrook *et al.*, Molecular Cloning--A Laboratory Manual (2nd ed.) Vol. 1-3, Cold Spring Harbor Laboratory, Cold Spring Harbor Press, NY, 1989, for as a reference to the known art.

A specific working example showing the isolation of phzO DNA from genomic DNA using probes comprising the PhzO coding sequences and intermediate hybridization conditions is provided in paragraph 222.

Thus, Applicants teach a polypeptide encoded by a nucleic acid sequence that hybridizes under medium stringency conditions.

The specification teaches polypeptides having PhzO activity which are encoded by nucleotide sequences 50% identical with SEQ ID NO:1

The Examiner alleges that: "The specification does not teach a polypeptide encoded by a sequence 50% or 60% identical with SEQ ID NO:1..." Applicants disagree with this allegation.

In paragraph 71, Applicants disclose exemplary methods for determining percent sequence identity. The methods include: "the GCG program package (Devereux, J., et al., Nucleic Acids Research 12(1): 387 (1984)), BLASTP, BLASTN, and FASTA (Altschul, S. F. et

al., J. Molec. Biol. 215: 403-410 (1990). The BLAST X program is publicly available from NCBI and other sources (BLAST Manual, Altschul, S., et al., NCBI NLM NIH Bethesda, Md. 20894; Altschul, S., et al., J. Mol. Biol. 215: 403-410 (1990) and Altschul et al., Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. 25: 3389-3402 (1997).), ALIGN (http://dot.imgen.bcm.tmc.edu:93- 31/seq-search/alignment.html), and Clusta1W (http://dot.imgen.bcm.edu:9331- /cgi-bin/multi-align/multi-align.pl) (Higgens, 1989)." Thus, the Applicants teach methods for determining percent sequence identity.

Similarly in paragraphs 156 through 212 Applicants disclose exemplary methods for the expression of peptides from isolated nucleic acid sequences. These methods are well known in the art. A working example of PhzO expression in transformed *E. coli* cells is provided beginning at paragraph 225.

Applicants have also provided ready assays to measure PhzO activity. For example, in paragraph 61 Applicants refer to methods for measuring phenazine production which are known in the art. In paragraph 62, lines 4-7, and in paragraph 231, Applicants disclose a fungal inhibition assay for measuring PhzO activity. In paragraph 239, Applicants provide a working example demonstrating the use of this assay. Furthermore, in paragraph 230, Applicants disclose an HPLC protocol for detection of metabolites that result from PhzO activity. In paragraph 238, Applicants provide a working example demonstrating the use of HPLC assays to confirm that strains that hybridize to the 2.1kb nucleotide sequence comprising the 1.5 kb phzO gene, do in fact possess PhzO activity.

The disclosed methods and assays can be used to distinguish nucleic acids with 50% sequence identity to SEQ ID NO:1 which encode polypeptides that have PhzO acitvity from among all nucleic acids with 50% homology to SEQ ID NO:1.

A skilled artisan who has mastered molecular biology techniques will know how to identify nucleic acids with 50% homology to SEQ ID NO:1, and will know how to assay the proteins produced by expression of these nucleic acids. The techniques required for the procedures, such as recombinant DNA technology, are well established and routinely used by those skilled in the art. Therefore, there would be no undue experimentation required to

distinguish nucleic acids that encode polypeptides having PhzO activity from all nucleic acids with 50% homology to SEQ ID NO:1.

The specification teaches how to identify polypeptides with 60% sequence identity to SEQ ID NO:2 which have PhzO activity, from all polypeptides with 60% sequence identity to SEQ ID NO:2.

The Examiner also raised the concern that there was insufficient guidance for one skilled in the art to practice the invention, because the specification allegedly does not show which amino acids of SEQ ID NO:2 can be altered and how, and which amino acids must not be changed in order to maintain activity of the protein. Applicant disagrees with these assertions.

As noted above, Applicants have provided methods known in the art for determining percent sequence identity. In addition, in paragraph 134, Applicants recite a specific method for determining the degree of identity between amino acids *i.e.,* the method of Pearson (Methods Enzymology 183:63-98, 1990). Also as noted above, Applicants have provided ready assays to measure PhzO activity.

Furthermore, in paragraph 92, Applicants disclose methods *e.g.,* alanine scanning mutagenesis, that can be used to determine which amino acids of a given polypeptide are essential for the experession of PhzO activity.

The disclosed assays can be used alone or in combination to distinguish polypeptides with 60% homology to SEQ ID NO:2 that have PhzO acitvity from among all those proteins with 60% homology to SEQ ID NO:2. A skilled artisan who has mastered molecular biology and protein biochemistry will know how to identify proteins with relevant stretches of homology, and will know how to assay the selected proteins. The techniques required for the procedures, such as recombinant DNA technology, are well established and routinely used by those skilled in the art. Therefore, there would be no undue experimentation required to distinguish polypeptides having PhzO activity from all proteins with 60% homology to SEQ ID NO:2.

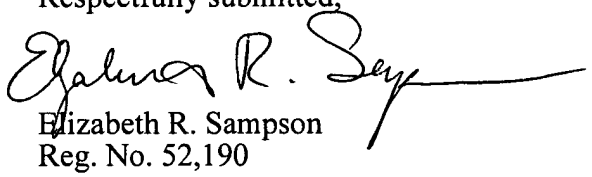
In summary, Applicants believe that the disclosure by the present application is sufficiently enabling for a person of ordinary skill in the art to practice the invention and the no undue experimentation is required. The rejection for inadequate enablement should thus be properly withdrawn.

CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 510-559-6066.

Respectfully submitted,



Elizabeth R. Sampson
Reg. No. 52,190

USDA, ARS, OTT
800 Buchanan Street
Albany, California 94710
Tel: 510-559-6066
Fax: 510-559-5736